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POTENCIES OF THE END BUD AND OTHER
CAUDAL LEVELS OF THE EARLY
CHICK EMBRYO, WITH SPECIAL
REFERENCE TO THE ORIGIN
OF THE METANEPHROS

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

By
CHARLES HAMILTON SEEVERS

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POTENCIES OF THE END BUD AND OTHER CAUDAL LEVELS OF THE EARLY CHICK EMBRYO, WITH SPECIAL REFERENCE TO THE ORIGIN OF THE METANEPHROS

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THREE FIGURES

INTRODUCTION

In 1926, Willier reported results of transplanting the entire pellucid area of young chick blastoderms to the chorio-allantoic membrane, to determine if gonads would differentiate in the absence of the primordial germ cells. In this, and in a subsequent detailed report (Willier and Rawles, '31) of transplants of whole blastoderms of presomite and early-somite stages, it was shown that all anterior structures back to and including the level of the mesonephros were differentiated in these grafts. In his studies on the rôle of Hensen's node in the formation of the chick embryo, Hunt ('31) has demonstrated that the isolated node level of the primitive-streak stage has almost as great capacity for differentiation as that shown by whole blastoderms of primitive streak, head-process and early-somite stages. There was no indication of differentiation of metanephros in either type of isolation, even in those from donors with twelve somites, the oldest stages used. The node level appeared to be totipotent for all of the embryo anterior to a certain level of the trunk, just in front of the metanephros. The failure of metanephros, the only criterion of differentiation of caudal levels of the embryo, to appear in these grafts led to the experiments outlined in this paper.

It was noted, especially in transplants of the node level, that the capacity to produce mesonephros increased as the node moved posteriorly. This was indicated by the greater frequency with which mesonephros was found in grafts from early-somite stages than in grafts from presomite stages. The presence of two additional structures, gonad and adrenal gland, in grafts of somite stages was also indicative that the node level acquires potentiality to form more posterior structures as it moves backward. On the basis of these results, it was predicted (Willier, '31) that, after the node had moved posteriorly to a certain level of the primitive streak, it would acquire the potency of metanephros also. This prediction was made with the reservation that certain other relations might be established, subsequent to those primarily set up by the node, which would be necessary for metanephros to differentiate.

About the eighteen- to twenty-somite stage, the node, which has become quite small, begins to increase in size very rapidly and forms, by the twenty-one- or twenty-two-somite stage, a prominent knob-like swelling, the end bud. On the basis of the preceding discussion, one might expect that the realization of capacity to form metanephros might accompany this change in the node.

Holmdahl ('26) has attributed considerable significance to the end bud. He believed that all of the embryo posterior to approximately the twenty-seventh or twenty-eighth somites is derived from material of the end bud, which he regarded as being composed of indifferent material. If this conception is true, we would expect metanephros to be formed from material proliferated from the end bud, since it lies in the region supposedly formed from this structure.

It was with this point in mind that experiments were carried out to determine the capacity of the end bud for differentiation of caudal structures. An analysis was first made, by means of chorio-allantoic grafts, of the differentiating capacity of the end bud and short primitive streak behind it; and, contrary to expectations, metanephros and, still more

surprising, neural tube and notochord did not differentiate in these grafts.

This led to a series of experiments to determine, if possible, what conditions must be set up in the posterior part of the embryo before metanephros will differentiate in grafts. Since the metanephros arises from two primordia, there exists the possibility of a dependent differentiation. Such a dependence had been postulated by Boyden ('28), whose experiments indicate that differentiation of the nephrogenous tissue is dependent on ureter formation. An analysis of this problem by transplantation of the metanephric primordia to the chorio-allantoic membrane supports such a conception.

It affords me much pleasure to express my sincere appreciation to Dr. B. H. Willier, to whom I am indebted for the suggestion of the problem and for helpful advice and criticism.

MATERIAL AND METHODS

The method used in these experiments was essentially that described by Willier ('24). After the donor eggs had been incubated to the desired age, the blastoderms were removed from the yolk and placed in a Petri dish containing warm saline solution. Before the desired levels were removed from the blastoderm, the exact stage of the donor, as indicated by the number of pairs of somites present, was determined. Great care was taken in making this somite count, since it was essential to know the exact stages of the donors used in all of the experiments. It was not sufficient to know the hourly age of the donors, for, as indicated in the tables presented in this paper, there is no very close relationship between age and somite number.

As soon as the stage of the donor had been determined, the level of the embryo desired for transplantation was isolated by carefully planned cuts. In the cases where the entire pellucid area of a certain level of the embryo was transplanted, the transections were made with a small iris-knife.

Finely ground steel needles were used to separate the minute metanephric primordia from the embryo.

The isolated part was then sucked up into a fine glass pipette, which had been drawn out at the tip to a diameter suited to the size of the implant. By means of the pipette, the graft was placed in contact with a blood vessel of the chorio-allantoic membrane of an eight- or nine-day host embryo. The host had been previously prepared by having a small rectangular window sawn in the shell over the blood vessel, which had been located by candling.

After a period of nine or ten days on the host membrane, the grafts were removed, fixed in Bouin's fluid, sectioned at 8μ , and stained with iron haematoxylin.

In connection with these experiments, a number of preparations of normal embryos were examined. These included whole mounts and serial sections of end-bud stages, with from eighteen to twenty-six somites, and serial sections of representative stages from this latter stage up to fifty-one somites. Besides my own preparations, I had the opportunity to examine a very fine set of serial sections of every somite stage between the eighteen- and fifty-one-somite stage, so kindly lent to me by Miss Mary Rawles, of this department.

White Leghorn eggs were used almost exclusively in these experiments.

DIFFERENTIATION CAPACITY OF GRAFTS OF END-BUD AND
PRIMITIVE-STREAK LEVELS OF THE EMBRYONIC
AXIS OF END-BUD STAGES

Transplants were first made of the end-bud and primitive-streak levels, with the idea that the former might possibly have the capacity to form metanephros. As has been previously pointed out, Hensen's node level of blastoderms up to the twelve-somite stage is not capable of differentiating into metanephros. It was believed, however, that it was not unlikely that the node level would acquire the capacity to form kidney after it had enlarged to form the end bud.

Before we proceed with an account of the experiments, it will be well to give a brief description of the events associated with the formation of the end bud in normal development. As the node moves posteriorly along the primitive streak, leaving in its wake notochord and the floor of the neural tube, as Wetzel ('29) has so clearly shown by his marking experiments, it becomes smaller and smaller, reaching its minimum size by the seventeen- or eighteen-somite stage. At this time the neuropore is still open, so that the node is partially enclosed by the presumptive neural-tube material, the rhomboidal sinus. Very soon after this stage, the node becomes a center of very active cell proliferation, and as a result forms a prominent knob-like swelling at the posterior end of the embryonic axis which is known as the end bud ('Nodus caudalis,' Rauber, '80; 'Schwanzknöpfchen,' Braun, '82; 'Rumpfknospe,' Keibel, '10; 'Endwulst,' Holmdahl, '24; 'Endknopf,' Wetzel, '29). End-bud formation is in progress by the eighteen- or nineteen-somite stage, and the end bud is fully formed by the twenty-two- or twenty-three-somite stage (fig. 1). Accompanying the enlargement of the node is the closure of the neuropore, so that the end bud is situated behind the closed neural tube, and the rhomboidal sinus is no longer apparent.

Behind the end bud is the short primitive streak which exists, as such, until the tail fold appears about the twenty-five-somite stage. At this time the end bud comes to lie at the most posterior end of the embryonic axis, the primitive streak turning in under as the folding occurs, and becoming so closely allied to the end bud as to lose its identity as a streak. From this time on, the end bud and the remnant of the primitive streak are situated at the tip of the developing tail bud.

It was not unreasonable to believe that an accompaniment of this sudden increase in activity on the part of the node might possibly be the acquisition of further potencies, especially those of the metanephros. This belief was strengthened by the fact that Holmdahl, Schumacher, and others have

attributed great significance to the end bud in the formation of the posterior part of the embryo.

To determine to what extent potencies are fixed in the end bud and primitive streak, levels of the blastoderm containing these structures were transplanted to the chorio-allantoic membrane. Transplants of three types were made: 1) the end-bud and primitive-streak levels; 2) the end-bud level alone; 3) the primitive-streak level alone.

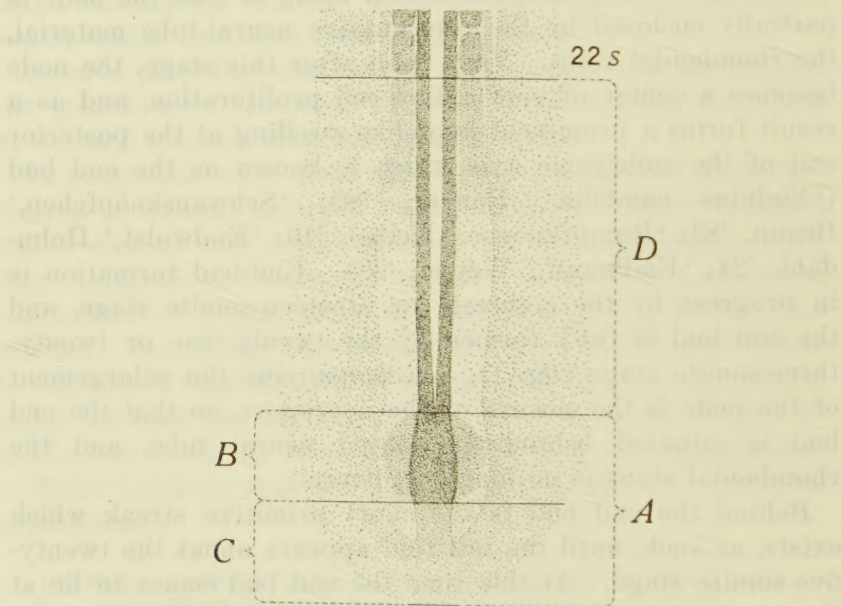


Fig. 1 Camera-lucida drawing of the posterior end of a twenty-two-somite embryo. The levels isolated, A, B, C, and D, are described in the text. The solid lines indicate where the transections were made.

I. Grafts of the level including both end bud and primitive streak

These grafts included all of the pellucid area of the end-bud and primitive-streak levels, indicated as level A, figure 1. Transplants were taken from donors varying from the seventeen-somite stage, at which time the node is at its minimum size before its enlargement to form the end bud, up to the

thirty-somite stage, when the end bud and primitive streak are at the tip of the developing tail bud.

The structures differentiated in these grafts are indicated in table 1. It is clearly shown that there was no differentiation of any structure characteristic of a specific level of the embryo. The only structures appearing were of a highly generalized type, found in grafts of any level and not indicative of any specific potentiality. Metanephros was not present in any case, and, still more unexpected, neural tube and notochord were not differentiated in a single graft.

With the exception of five of the twenty-three grafts in which there appeared a few scattered ganglion cells, there was only one graft (127-8), from an eighteen-somite donor, in which there was indication of nervous tissue. In this graft there was a small area of nervous tissue which was not organized into a neural tube with a lumen and epithelium, and was not accompanied by notochord. It is probable that in this case a small amount of rhomboidal sinus was included with the material isolated, for at this stage the node is to some extent bounded laterally by presumptive medullary plate. An effort was made, at the time of isolation, to include as little of this material as possible. In stages later than this, the end bud lies posterior to the closed neural tube, and this difficulty was not encountered. The presence of ganglion cells in five of the grafts appears to have little significance, since these cells appear in grafts of many levels in which there is no evidence of central nervous system.

As noted in the table, gut, smooth muscle, and integument appeared most frequently, while cartilage, skeletal muscle, and feather germs were present in less than one-third of the cases. The grafts were all quite small and in many cases the bulk of the graft was mesenchyme, the differentiated tissues comprising only a small part. Differentiation was usually comparatively poor, i.e., the gut which appeared in these grafts, for example, was not characteristic of gut which appears in posterior levels. The largest grafts obtained from this level were no more than 4 mm. in length. This is approxi-

mately one-sixth to one-eighth of the size of the grafts obtained by Hunt ('31) from isolations of Hensen's node level of primitive-streak stages.

Successful grafts were obtained from twenty-three of the forty-four hosts which remained alive up to the time of removal of grafts. In the other twenty-one cases, the grafts failed to become incorporated or were so poorly incorporated as to contain only mesenchyme. It is of interest to note that the percentage of successful grafts (52 per cent) is considerably lower than that obtained by Hunt from transplants of the node of primitive-streak stages (73 per cent) and of head-process stages (60 per cent).

II. Grafts of the end-bud level alone

The level B, figure 1, was isolated to see if the end-bud level differed in capacity for differentiation from the primitive-streak level behind it. Since the end bud and primitive streak together had shown so little capacity, we hardly expected different results in this case. The results of these transplantations are so uniform, and agree so well with the previous experiment, that it is unnecessary to arrange them in tabular form.

With the exception of one case, axiate structures were not present. In this one graft (DD-4), from a seventeen-somite donor, some rhomboidal sinus material was intentionally included with the node, and as a result well-defined neural tube and notochord were present. In the other grafts special care was taken not to include any of the presumptive medullary material.

Eleven grafts of this level, from donors varying from seventeen- to twenty-five-somite stages, were sectioned and examined. These eleven grafts were obtained from twenty-nine hosts which were alive at the end of the experimental period, showing only 38 per cent success. The grafts were very small and the differentiation was limited, for the most part, to small areas of gut, smooth muscle, and integument. Nine of the eleven had gut and muscle, the other two consist-

ing of integument only. The single case in which cartilage appeared was in the afore-mentioned DD-4. Integument appeared seven times and feather germs twice in the eleven grafts. It is apparent, therefore, that this level has the capacity to produce only gut, smooth muscle, and integumentary structures.

III. Grafts of the primitive-streak level alone

Isolations were made which included all of the pellucid area posterior to the end bud, indicated as level C, figure 1. There were eleven successful grafts, taken from seventeen-somite up to twenty-five-somite donors, resulting from the twenty one cases in which the hosts were alive (52 per cent successful). The results are very little different from those of the end bud level. Four of the grafts have nothing but integument, one of the four having a few feather germs. Five showed differentiation of gut, only three of which have muscle associated with it, while two of the five had skin present. One graft showed only muscle, while the eleventh had muscle and skin. Cartilage was not present in any case.

EXPERIMENTS CONCERNING THE DIFFERENTIATION OF THE METANEPHROS

It has been shown that metanephros does not differentiate in grafts of Hensen's node or primitive-streak levels of the early chick blastoderm (Hunt, '31); in grafts of whole blastoderms up to twelve-somite stage (Willier and Rawles, '31); or in grafts of the end-bud and primitive-streak levels up to the thirty-somite stage. At what stage, then, is it possible to obtain differentiation of metanephros in grafts, and what are the factors that determine its differentiation? As suitable conditions apparently do not exist in the node or end-bud levels, it seemed likely that differentiation would result from transplantation of either, 1) the region between the last differentiated somite and the end bud, or 2) the level of the embryo in which the primordia of the metanephros arise.

It is necessary to give a short account of the normal development of the metanephric primordia before we proceed with the analysis. The metanephros forms from two primordia, the secreting tubules and renal corpuscles arising from the nephrogenous tissue of the thirty-first, thirty-second, and thirty-third somites, while the ureter and collecting tubules are formed from the metanephric diverticulum—an evagination from the wolffian duct at its posterior end. The wolffian duct, arising in the anterior part of the body, grows posteriorly, usually keeping pace with the formation of somites, until it reaches the level of the cloaca. It reaches its most posterior extent about the thirty-five somite stage, and enters the cloaca at the level of the thirty-fourth or thirty-fifth somite, about the forty-somite stage. The metanephric diverticulum is formed from the wolffian duct near its point of union with the cloaca, and grows anteriorly where it becomes closely associated with the metanephrogenous tissue. Differentiation of this metanephric blastema into secreting tubules and renal bodies takes place following its contact with the metanephric diverticulum which is differentiating into the collecting elements of the kidney.

It is evident that to include both primordia of the kidney in an isolated level of the embryo, it is necessary to have the region between the thirty-first and thirty-fifth somites, inclusive. Following the failure of metanephros to develop in grafts of the region between the last differentiated somite and the end bud, which were made as the first step in the analysis of the factors necessary for the differentiation of the metanephros, grafts were made of the thirty-one- to thirty-five-somite level, inclusive. A third series of experiments was then made, in which the metanephrogenous tissue, together with the section of the wolffian duct from which the metanephric diverticulum arises, were isolated and transplanted. The results of these three series of experiments are given below.

I. Grafts of the level between the last differentiated somite and the end bud

These grafts were made in view of the possibility that the presumptive metanephric material might be located in the region in front of the end bud, and be laid down by elongation of this region instead of being formed from the material of the end bud. If the segmental plate should elongate to any extent during somite formation, more somites would be formed from it than one would estimate by measuring it at a given stage.

Grafts were made of all of the pellucid area between the last differentiated somite and the end bud (level D, fig. 1) from donors ranging from eighteen somites to twenty-six somites. If no elongation of this region occurs during somite formation, it is estimated that it would form approximately six somites. Even if only six somites form from this region, it is seen that some of the presumptive metanephric level will be formed from this region of the older donors used.

Table 1 indicates the structures differentiated in the fifteen successful grafts obtained from twenty-five hosts. Only those structures that one would expect from a graft of this level were found. Metanephros did not occur in any case. Mesonephros was present in eleven of the fifteen grafts, and there was no question as to its identity, thus ruling out any doubt as to the presence of metanephros.

It is pertinent at this point to discuss the differences existing between meso- and metanephros by which we were able to differentiate between the two in grafts. While there are a number of minor differences, which taken as a whole are convincing enough, our final criterion of metanephros was the presence of a ureter which was found to be in union with the collecting tubules. In the case of mesonephros, the wolffian duct was always in connection with the collecting tubules.

It was found upon examination of grafts of the entire wolffian ridge of five-day donors, in which both metanephros and mesonephros occurred, that we could distinguish a very characteristic difference in the histology of the wolffian duct

and ureter. The wolffian duct has a very distinct, high columnar epithelium at all levels. Near the point where the wolffian duct connects with the collecting tubules, the epithelium is simple columnar, the cells being very high. At other levels, the wolffian duct is characterized by a columnar epithelium in which the nuclei are arranged in several layers. The epithelium of the ureter, on the other hand, is very rarely columnar at any level. Near its point of division to form collecting tubules, the ureter has simple, cuboidal epithelium. As we follow the ureter toward the cloaca, we seen that its epithelium becomes stratified, but is never columnar, being, instead, a so-called transitional type. The epithelium of the ureter is thrown into folds so that the stratification varies in thickness, in any cross section, from one cell to five cells. The epithelium of the wolffian duct, on the contrary, is of a uniform thickness at any particular level. The epithelium of the ureter is surrounded by a wide band of flat cells which form the tunica propria. This band is, in most cases, considerably wider than that found about the wolffian duct.

By means of these features, we were able to distinguish readily between ureter and wolffian duct in our grafts, and so tell with certainty which type of nephric tissue was present. Besides this criterion, there were others by which we could distinguish one type from another. The tubules of the mesonephros are usually less closely bound together by interstitial tissue, giving the mesonephros the appearance of being much less compact than the metanephros. This furnishes a difference which is usually readily apparent. The tubules and glomeruli of the metanephros are uniformly smaller in size than those of the mesonephros. Metanephros, at this stage, usually contains a considerable amount of undifferentiated nephrogenous tissue, a feature never apparent in the case of mesonephros. On the basis of these differences, we feel justified in stating whether meso- or metanephros was present in any particular graft.

Besides mesonephros, we found neural tube, notochord, gut, muscle, cartilage, skin, and feather germs in a large majority

of the grafts of this series. Gonad and adrenal glands were found in only two grafts, which is a rather low percentage, considering that these bodies normally arise at this level.

II. Grafts of the level of the blastoderm in which the metanephric primordia normally arise

Since metanephros did not form in any of the previous types of grafts, we now turned to the level of the embryo in which the primordia arise. We may divide the series of grafts, listed in table 2, into two groups, 1) those from donors younger than the thirty-five-somite stage; these grafts from isolations including the presumptive metanephrogenous tissue and a section of the wolffian duct, but not the specific level of the duct which gives rise to the ureter; 2) the thirty-one- to thirty-five-somite level, which includes both the metanephrogenous tissue and the specific ureter-forming level of the wolffian duct.

The results of these experiments are so uniform as to render it unnecessary to consider these two groups separately from this point on. A total of thirty-three grafts were sectioned and examined. These were taken from donors between the twenty-seven- and fifty-one-somite stages. The grafts were quite large in most instances, since part of the hind-limb level was included, this resulting in large sections of limb cartilage and bone. Since we are concerned only with the metanephros, it will suffice to say that the grafts up to the fifty-somite-stage showed, uniformly, differentiation of only neural tube, notochord, cloaca, cartilage, bone, integument, feather germs, smooth and skeletal muscle. We first identified cloaca by the fact that wolffian ducts and ureters joined with it. We had no other way of distinguishing it from hind gut in the grafts. The epithelium was of a stratified columnar type, which was non-specific as far as we could tell. Mesonephros was found in eight of the grafts. In every case, the mesonephros consisted only of a few tubules, which were, however, so evidently mesonephric as to leave no doubt as to their identity, using the criteria already described. This differenti-

TABLE 2

Structures differentiated in grafts of the level of the embryo in which the metanephric primordia arise; and in grafts of the metanephric primordia

SERIAL NUMBER OF GRAFT	LEVEL OF THE BLASTODERM GRAFTED	STAGE OF DONOR (SOMITE NUMBER)	AGE OF DONOR (HOURS)	MESONEPHROS	WOLFFIAN DUCT	METANEPHROS				CLOACA	SPINAL CORD	NOTOCHORD	CARTILAGE	BONE	INTEGUMENT	FEATHER GERMS	MUSCLE
						Secreting tubules	Glomeruli	Collecting tubules	Ureter								
F-1	Posterior to 27s.	27	60						+								
J-6	Posterior to 29s.	29	61							+							
C-2	Posterior to 29s.	29	59							+		+					
E-8	Posterior to 30s.	30	64							+		+					
G-11	Posterior to 29s.	31	64							+		+					
B-2	Posterior to 31s.	31	61							+		+				+	
J-3	31s. to 32s. (inclusive)	32	60							+							
C-7	Posterior to 30s.	33	60							+		+					
K-2	31s. to 33s. (inclusive)	33	60							+		+		+			
J-10	30s. to 34s. (inclusive)	34	61							+		+					
F-9	30s. to 33s. (inclusive)	34	63		+	+				+		+					
D-1	Posterior to 30s.	35	60							+		+		+			
J-5	31s. to 35s. (inclusive)	35	61							+		+		+			
H-6	30s. to 33s. (inclusive)	37	63		+	+				+		+		+			
AA-2	30s. to 35s. (inclusive)	40	69		+	+				+		+		+			
Z-9	30s. to 35s. (inclusive)	40	70		+	+				+		+		+			
X-7	30s. to 38s. (inclusive)	42	71		+	+				+		+		+			
X-8	31s. to 38s. (inclusive)	42	71							+		+		+			
Z-7	30s. to 35s. (inclusive)	43	70		+	+				+		+		+			
O-5	30s. to 35s. (inclusive)	44	72							+		+		+			
117-4	31s. to 34s. (inclusive)	45	84		+	+				+		+		+			
122-1	29s. to 35s. (inclusive)	45	93							+		+		+			
122-3	31s. to 35s. (inclusive)	46	93							+		+		+			
117-5	31s. to 35s. (inclusive)	46	84		+	+				+		+		+			
125-1	31s. to 35s. (inclusive)	47	94							+		+		+			
125-2	31s. to 35s. (inclusive)	47	94							+		+		+			
122-4	31s. to 35s. (inclusive)	48	94							+		+		+			
122-5	31s. to 35s. (inclusive)	48	94							+		+		+			
122-6	31s. to 35s. (inclusive)	48	94							+		+		+			
126-3	31s. to 35s. (inclusive)	49	95							+		+		+			
BB-4	30s. to 34s. (inclusive)	50	94			+	+	+	+	+		+		+			
BB-3	30s. to 34s. (inclusive)	51	95			+	+	+	+	+		+		+			
BB-6	30s. to 35s. (inclusive)	51	95			+	+	+	+	+		+		+			
QQ-6	M (left)	36	74	+	+					+							+
LL-3	M (left)	40	72							+							+
MM-2	M (left)	40	73	+	+					+							
QQ-8	M (right)	42	75							+							+
111-3	M (right)	45	90							+							
104-1	M (right)	46	92		+					+							
103-2	M (right)	46	92							+							+
103-1	M (left)	47	93		+					+							+
101-1	M (left)	48	93		+					+							+
109-7	M (right)	48	95		+					+							
106-2	M (right)	49	94		+					+							
111-2	M (left)	50	94							+							+
107-4	M (left)	50	98		+	+	+	+	+	+							+
106-4	M (left)	51	98			+	+	+	+	+							
107-2	M (right)	51	99							+							+
107-1	M (right)	51	98							+							

ation of mesonephros may have been due to the fact that there probably is not a sharp boundary between meso- and metanephros, at least the nephrogenous tissue of the transition zone may not have its potency strictly limited. The nephrogenous tissue of the proximity of the thirtieth somite may not form any mesonephric tubules in normal development, yet have the potentiality to do so in grafts.

There was not a single indication of metanephros in any of the grafts from donors younger than the fifty-somite-stage (ninety-four hours' incubation). However, in the single graft from a fifty-somite donor, and in the two from fifty-one-somite donors, there was very fine differentiation of metanephros and all of its component parts. Differentiation of kidney in these cases was complete in every way, there being well-defined secreting tubules and glomeruli, as well as collecting tubules and ureter which entered into the cloaca. We have described, in the preceding section of the paper, the histological appearance of the different levels of the ureter. By following the branches of the ureter, we were able to ascertain which were collecting tubules. The tubules which were presumed to be secreting tubules were first identified by their close association with the epithelium of the renal corpuscles. Upon examination, we found that histological differences were present, even at this stage, by which we could determine whether both types were present. The collecting tubules were found to have a simple cuboidal epithelium, which was lower than that of the secreting tubules, which was generally pyramidal or low columnar. The cytoplasm of the collecting tubules was quite clear, in contrast to the granular nature of the cytoplasm of the secreting tubules, which stained considerably darker. In the proximity of the secretory elements there was usually some undifferentiated nephrogenous tissue.

It is striking that complete differentiation of metanephros should appear in the grafts from fifty- and fifty-one-somite donors, while there was no indication of any part of the kidney in grafts of any level from a younger donor. One might expect that the metanephrogenous tissue would differentiate

even though the ureter was not present, but apparently it does not. After the ureter is formed, this tissue might differentiate even though the ureter was absent in the graft, since there is the possibility that the age of the tissue is the factor necessary for differentiation.

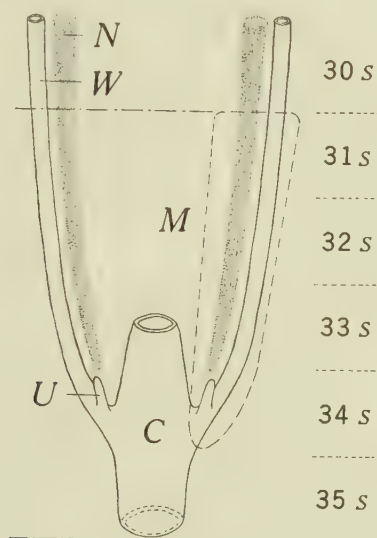


Fig. 2 Diagram of the metanephric primordia as they appear in a fifty-one-somite chick. The isolations which were made, namely, the thirty-one- to thirty-five-somite level and metanephric primordia (*M*), are described in the text. The extent of isolation *M* is indicated by the dash line. *C*, cloaca; *N*, nephrogenous tissue; *U*, ureter; *W*, wolffian duct.

III. Grafts of the isolated metanephric primordia

In order to confirm the results of the experiments just described, a series of grafts were made of the metanephrogenous tissue together with the wolffian duct posterior to the thirtieth somite, from one side only (*M*, fig. 2). In this way, the specific ureter-forming level of the wolffian duct and the nephrogenous tissue were transplanted together. The wolffian duct and the nephrogenous tissue are in very close proximity, and the metanephric diverticulum and nephrogenous tissue are in even closer association immediately after the

former arises. Although it is realized that transplantation of the two primordia separately would constitute a critical experiment, we found that their close association rendered separation impractical.

In order to insure that all of the posterior part of the wolffian duct would be included, a small part of the cloaca was removed with the wolffian duct, as is evidenced by the appearance of cloaca in most of the grafts. This type of isolation is designated as *M*, figure 2. The results of these grafting experiments are given in table 2, the laterality of the isolation being indicated in each case. The isolations were very small and the grafts were correspondingly small. The percentage of successful grafts was very low, only sixteen (35 per cent) grafts were obtained from the forty-six hosts which survived the experimental period.

The results of these grafts confirm those of the preceding experiment in every way. Metanephros did not differentiate in the eleven grafts from donors between the thirty-six-somite stage and the forty-nine-somite stage. In one of the two grafts from fifty-somite donors, and in one of the three grafts from fifty-one somite donors, there was excellently differentiated kidney. A photomicrograph of a section of graft 107-4, which was from a fifty-somite donor, is shown in figure 3. This section is typical of all of the sections of the graft, in that the graft is made up entirely of metanephros, except for a short section of wolffian duct and cloaca. This section is through the level of the graft in which the ureter is branching into smaller tubes which form collecting tubules. The ureter enters the short section of wolffian duct which is in the graft at another point. The nephrogenous tissue apparently collected in masses about the ends of the secondary branches of the ureter and there differentiated into secreting tubules and renal bodies. There is still evidence of undifferentiated nephrogenous tissue accompanying the differentiated tubules. We see, then, that transplantation of the metanephric primordia, at a time when both are definitely indicated and in contact, yields a complete, though minute, kidney.



Fig. 3 Photomicrograph of a section through graft 107-4, from an implant of the left metanephric primordia of a fifty-somite embryo. The entire graft is composed of kidney tissue. This section is taken at the level where the ureter is breaking up into collecting tubules. $\times 125$.

DISCUSSION

It is shown by the above experiments that the material of the end-bud and primitive-streak levels of the blastoderm, during end-bud stages, has very little capacity for differentiation of structures other than those of the most generalized type, i.e., gut, smooth and skeletal muscle, cartilage, skin, and feather germs. The end-bud level alone and the primitive-

streak level alone apparently do not have the capacity to produce skeletal muscle and cartilage. The absence of neural tube and notochord offered the most unexpected result, especially in view of the manner in which these axial structures are believed to develop in the posterior part of the normal embryo. The failure of metanephros to differentiate in grafts of these levels is more easily accounted for, in view of the results of the subsequent experiments in which the metanephric primordia were transplanted.

We wish to consider, first, the failure of axial structures to appear in grafts of the end-bud level, and the bearing of this on the problem of the significance of the end bud in the formation of posterior structures of the embryo. The apparent lack of capacity of the end-bud level to form neural tube and notochord is in marked contrast to the capacity of the level containing Hensen's node of presomite and early-somite stages, to form these structures (Hunt, '31). The node level apparently loses its capacity to form neural tube and notochord just before the time of end-bud formation. The node level no longer has the capacity to form neural tube after the disappearance of the rhomboidal sinus (presumptive medullary plate). Wetzel ('29) has shown by his marking experiments that only the floor of the neural tube forms directly from the node material, while the walls and roof are derived from the presumptive medullary-plate material lateral to the node. It seems quite reasonable that all of the neural tube formed up to the time that the end bud first appears and neuropore closes, be laid out as presumptive material in the rhomboidal sinus, since neural tube differentiated in grafts in which a portion of the rhomboidal sinus was included.

If it be true that the presumptive medullary plate for a certain part of the body is laid out in this manner, what, then, is the source of the neural-tube material after the neuropore closes and the rhomboidal sinus has disappeared? Considerable evidence has been presented in favor of the view that the neural tube of the posterior levels forms exclusively

through differentiation of indifferent cells of the end bud and does not arise by the closure of ectodermal neural folds.

This was first noted in the parrot and in the chick by Braun ('82), who recognized two types of neural-tube formation, the first, by a closure of ectodermal folds; the second, in the posterior extremity, from a solid anlage, the 'Medullarstrang,' which becomes hollowed out secondarily. Keibel and Elze ('08) state that in the human embryo the caudal end of the medullary anlage arises not through the growing together and closure of medullary folds, but differentiates with chorda from an indifferent cell mass, the tail bud.

This idea was not developed any further until Holmdahl ('25), from his studies on bird and mammalian embryos, applied it as strong evidence for his theory of primary and secondary development, and Schumacher ('27) described the process very carefully in the chick. Holmdahl believed that all of the neural tube and notochord of the posterior half of the body forms directly from the end bud by a process of canalization of the solid strand. His observations of sections of normal chick and mammalian embryos indicate that there is a gradual transition from the end bud, in which the cells are irregularly arranged in a compact mass; through a stage in which a thinning out in the central part produces a lumen with an epithelium; to the definitive neural tube, an oval, epithelial tube with a dorsoventrally elongated lumen. He conceived of this process being initiated at the time of the closure of the neuropore and the formation of the end bud.

Schumacher's ('27) observations were made in a search for an interpretation for a so-called, teratological condition found by a number of investigators in the caudal levels of the neural tube of bird and mammal embryos. This condition, known as 'multiplicity of neural tubes,' in which there was more than one lumen in the spinal cord, was believed to be an abnormal situation and had been described many times as such, and had even been produced experimentally in the chick by Waelsch ('14) and Gawrilenko ('24). Schumacher has shown, however, that this is not an anomalous situation,

but, in reality, is the normal method of formation of the neural tube in caudal levels. He described and figured (figs. 1 to 6, pp. 84, 86, '27) sections from a normal fifty-seven-hour chick embryo, showing very clearly how differentiation of neural tube and notochord takes place from the end-bud material. During the thinning-out process, there is apparent, in most cases, more than one lumen, which, as differentiation progresses, coalesce to form the single lumen of the definitive neural tube. The notochord is not at first sharply delimited from the medullary strand, but soon separates from it, as neural-tube differentiation progresses.

There seems to be no doubt that this is the usual method of formation of axial structures in the caudal end of the embryo. I have examined a number of series of sections of chick embryos, varying from the twenty-one-somite stage to the fifty-one-somite stage and they all seem to show that these observers were correct in their interpretation that the neural tube and notochord form from the end bud directly, as described.

Since it appears so evident that the neural tube forms from the end bud, it is of interest to observe that this level does not differentiate in the grafts into nervous tissue and notochord. These results indicate that the cells of the end bud are physiologically indifferent. The material may be destined to form these structures, but evidently does not have sufficient organization to give rise to axiate organs. At the time the isolations were made, the end-bud material does not have capacity to self-differentiate structures which are normally traced to it, since only generalized tissues differentiate from it. Holmdahl ('26) and Schumacher ('27) speak of the end bud as being indifferent, apparently in the morphological sense of being histologically undifferentiated, Holmdahl characterizing it as an 'indifferente Zellenmasse,' a 'Sprossungszentrum' for the caudal levels of the embryo. It seems that this interpretation of the end bud is a correct one, since it evidently is a proliferation center for material of the caudal neural tube and notochord, yet is supplying material with a

low grade of determination. That the end bud is a center of marked activity has been demonstrated by Hyman ('27), who employed the method of differential susceptibility to toxic agents. The 'tail bud' was found to be the most active region of the two-day chick. From this, one would expect that the end bud would have a higher degree of determination, but evidently this is not so.

According to Wetzel ('29), the end bud is the same in form and material as the node and anterior part of the streak of earlier stages, namely, the place of formation of notochord, neural tube, and somites. This may be true, yet the end bud differs radically from the node of early stages in that the node is a center of organization possessing a marked degree of determination (Hunt, '31).

Holmdahl ('25) arrived at essentially this same conclusion largely through observational evidence, and formulated from it his theory of two phases in the development of the embryo. He expressed the view that all of the embryo posterior to the midtrunk level forms from indifferent material of the end bud (secondary body development), in contrast to the formation of the head and anterior half of the trunk from determined germ-layer material laid down as the node moves posteriorly along the primitive streak (primary body development).

Holmdahl estimated that the posterior limit of primary development is located about the level of the twenty-seventh or twenty-eighth somite. This estimation was based on his belief that all of the material laid out in front of the end bud at the time of its formation, is the product of primary development. Since the end bud is formed at the twenty-one to twenty-three-somite stage, and since there is segmental plate at this time capable of forming five or six somites, if no elongation occurs, Holmdahl has estimated that about twenty-seven or twenty-eight somites are formed during primary development. Holmdahl performed a set of experiments which entailed production of a lesion by electrocautery at the posterior end of the differentiated embryonic axis, just in front of the end bud, of a forty-eight-hour chick. Two days

later, the lesion was found in the trunk region, just behind the wing buds. He interpreted this to mean that the level behind the lesion was formed largely from end-bud material, and that the boundary between the two developmental phases is in the lumbosacral region.

Gräper ('27) has criticized this experiment on the ground that the embryos were not vitally stained before the lesion was made, so that there was no certainty as to where the marks were made. Gräper was of the opinion that the end bud does not play such an important part in body formation, but that a major portion of the caudal levels arises by elongation and growth of material in front of the end bud.

The posterior boundary established by Holmdahl as the extent of primary development from determined material, is in very close agreement with the posterior extent of structures differentiated in grafts of the node of presomite and early-somite stages (Hunt, '31) and in grafts of whole blastoderms of the same stages (Willier and Rawles, '31). Mesonephros was the most posteriorly situated organ found in grafts of the node level or whole blastoderms of presomite stages. Mesonephros is found between the fifteenth and thirtieth somites, but we have no evidence that all levels of the mesonephros were represented in these grafts. We see no very good reason, on the other hand, why the posterior levels of the mesonephros were not present, except that gonad, which is normally in close association with mesonephros in the region between the twenty-first and twenty-sixth somites, was not present in these grafts. Gonads did appear, however, in grafts of whole blastoderms of early-somite stages, so it is believed that all levels of the embryo back to about the thirtieth somite are determined by the time somites begin to appear. The single criterion of a level caudal to the thirtieth somite, namely, metanephros, is missing from these grafts. So we see that these results agree fairly well with Holmdahl's theory that structures back to about the twenty-eighth somite are formed during primary development from determined material.

The transplants of the end-bud level of the embryo, described above, while confirming the idea that the end bud is made up of undetermined material, do not throw much light on the question of whether the end bud actually contributes the material for the formation of caudal parts, which Holmdahl has postulated as the second phase of the developmental process. We are convinced that he is correct in his interpretation that neural tube, notochord, somites, etc., of caudal levels are formed from the indifferent cells of the end bud. We have already discussed the evidence that neural tube and notochord are formed from the end bud. It appears that Holmdahl's conception of two phases in the development of the embryo is justified, since there is such a marked difference in the time of determination of the structures of the anterior and posterior levels of the body. Neural tube, notochord, and metanephros are not determined in the caudal levels until the time that the rudiments of these organs are about to appear, while the potencies of structures of anterior levels are determined long before the time of histological differentiation, as evidence by the determination of mesonephros in the primitive-streak stage. That the material proliferated by the end bud actually acquires specific potency for neural tube and notochord is indicated by four grafts, not previously mentioned, of the tail bud, which included besides the end bud and remnant of the primitive-streak mass beneath it, a short section of neural tube and notochord which had formed from end bud material. These grafts—one (N-5) from a twenty-nine-somite donor, two (J-1, J-14) from thirty-one-somite donors, and one (O-1) from a thirty-three-somite chick—differentiated into neural tube, notochord, gut, smooth muscle, cartilage, skeletal muscle, skin, and feather germs. This result indicates that as soon as the rudiments of the neural tube and notochord are formed, the potencies become fixed.

It is of interest in this connection to point out that Bijtel ('31) has recently contributed some evidence in connection with this problem in urodeles and anurans. As a result of

his vital-staining experiments, he is of the opinion that the organs of the tail of these larvae do not form from indifferent cell material of the tail bud, but that the neural tube and notochord are formed by elongation of the anlagen of these organs following the medullary-plate stage.

We wish to consider, now, the problem of the differentiation of the metanephros. We have shown that metanephros does not form in grafts taken from donors younger than the fifty-somite stage. Willier ('30, p. 208) had previously obtained metanephros in grafts of the entire wolffian ridge of five-day embryos. That metanephros does not form earlier than just indicated was found to be true in grafts of the level of the embryo in which the metanephric primordia arise, and in grafts of the metanephrogenous tissue together with the section of the wolffian duct that gives rise to the ureter.

Since the fifty- or fifty-one-somite stage is the earliest stage in which both primordia (metanephrogenous tissue and metanephric diverticulum) are definitely indicated, we seem justified in the conclusion that both primordia must be present in the graft at the time of its isolation. While not positive that the metanephric diverticulum was present in these grafts at the time the isolation was made, we infer so from studies of preparations of normal embryos which showed definitely that the diverticulum is present in fifty- or fifty-one-somite embryos.

It seems that the wolffian duct apparently does not have the capacity to form a ureter in grafts. We may rule out the possibility that ureter formation is dependent on nephrogenous tissue, since nephrogenous tissue is present in all of the grafts from stages later than thirty-five somites in exactly the same relationship to the wolffian duct as it bears in normal development, yet ureter does not arise in these grafts. In many of the grafts in which metanephros did not occur, the specific ureter-forming level of the wolffian duct was present, yet the ureter seems to have been inhibited from forming. Boyden ('28) has presented evidence that the wolffian duct must enter the cloaca before it will form a ureter, but in all

of these grafts from embryos with over forty somites, the wolffian duct had entered the cloaca, yet no ureter develops. Lillie ('04) destroyed varying amounts of the posterior end of twenty-nine-somite chick embryos by cauterization, and found that kidneys and ureters were absent in the defective embryos which resulted. Since the metanephric level of the wolffian duct was destroyed by the operation, he postulated that ureter formation is either limited to a short region of the wolffian duct or is dependent on a certain amount of kind of development of the allantois or cloaca.

The fact that nephrogenous tissue will not differentiate until the ureter appears, suggests that differentiation of the former is dependent on the formation of the ureter. We noted before that in every case in which differentiation of metanephros occurred, all of the component parts were present. The presumptive metanephrogenous tissue was present in all of the grafts from stages over the thirty-five-somite stage, yet did not differentiate independently of the ureter. There was no indication of independent acquisition of potencies of secreting tubules and renal corpuscles, unless the nephrogenous tissue acquired these specific potencies simultaneously with the appearance of the ureter—a possibility which is not very likely. The most critical experiment to test the capacity of nephrogenous tissue to differentiate independently, would be to separate the two primordia just after the ureter had appeared, but, as we have pointed out previously, their close approximation makes this impractical.

That the differentiation of the nephrogenous tissue is dependent on the presence of the metanephric diverticulum, is in agreement with the view expressed by Boyden ('28) as a result of his experiments in which he prevented the posterior growth of the wolffian duct in the chick. As the wolffian ducts did not enter the cloaca in these cases, presumably, the ureters failed to appear, and no secreting tubules and renal bodies were formed. There was a concentration of nephrogenous tissue medial to the posterior cardinal veins, but this blastema never differentiated into secreting tubules and renal cor-

puscles. Boyden ('32) also reports a 10-mm. human embryo in which the left wolffian duct failed to descend below the level of the stomach, resulting in the absence of the left ureter. The left metanephric blastema was fused to the right kidney, but did not differentiate into tubules.

Hoadley ('26), on the contrary, presented evidence that in the case of mesonephros, the secreting elements were determined first and appeared in grafts from younger donors than did the collecting tubules and wolffian duct. This would indicate independent origin and differentiation of the secreting and collecting elements of the mesonephros.

As to the nature of the action of ureter on the nephrogenous tissue in the metanephros, these experiments offer no suggestion.

SUMMARY

1. The end-bud and primitive-streak levels of the embryonic axis of end-bud stages of the chick embryo have the capacity to form only generalized, non-axiate structures in chorio-allantoic grafts. Neural tube, notochord, and metanephros do not differentiate in these grafts.

2. Evidence is presented to show that neural tube and notochord of caudal levels normally form directly from the end bud, the former by the canalization of a solid strand instead of by closure of ectodermal neural folds. Even though these structures are traced to the end bud, the grafting experiments show that the latter does not have specific potency for neural-tube and notochord formation.

3. The end-bud level is, therefore, shown to be a physiologically indifferent cell mass, in contrast to Hensen's node level of presomite stages, which has been shown to have capacity for differentiation of almost all structures of the anterior half of the embryo.

4. Structures of caudal levels—neural tube, notochord, and metanephros—are not determined until the material is actually incorporated in the rudiments of these organs, while structures of the body anterior to approximately the twenty-sixth to thirtieth somite level are determined as early as the

primitive-streak and head-process stages. Since there is such a marked difference in time of determination of structures of anterior and caudal levels, Holmdahl's theory of two phases in the development of the embryo seems justified.

5. Grafts of the prospective metanephric level (thirty-one to thirty-five somites, inclusive), and grafts of the isolated metanephric primordia from fifty- and fifty-one-somite embryos, differentiated into kidney which was complete in every respect, while grafts of the same types from younger donors did not form metanephros in any case. Since this is the earliest stage in which the ureter is present and in contact with the metanephrogenous tissue, it is believed that this condition must exist before metanephros will differentiate.

6. As the metanephrogenous tissue did not in any case differentiate independently of the ureter, even though present in many grafts in which the metanephric diverticulum was absent, it is suggested that the differentiation of the metanephric blastema is dependent on ureter formation.

LITERATURE CITED

- BIJTEL, J. H. 1931 Über die Entwicklung des Schwanzes bei Amphibien. Arch. f. Entw., Bd. 125, S. 448-486.
- BOYDEN, E. A. 1922 The development of the cloaca in birds. Am. J. Anat., vol. 30, pp. 163-202.
- 1924 Experimental study on the cloaca and allantois. J. Exp. Zool., vol. 40, pp. 437-471.
- 1928 Experimental obstruction of the metanephric ducts. Proc. Soc. Exp. Biol. and Med., vol. 24, pp. 572-576.
- 1932 An inquiry into the cause of congenital absence of kidney. Abstract of paper before the American Association of Anatomists. Anat. Rec., vol. 52, p. 5, Supplement.
- BRAUN, M. 1881 Die Entwicklung des Wellenpapageis (*Melopsittacus undulatus* Sh.). S. 145-164. Würzburg.
- 1882 Entwicklungsvorgänge am Schwanzende bei einigen Säugtieren mit Berücksichtigung der verhältnisse beim Menschen. Arch. f. Anat. u. Phys., Anat. Abt., S. 207-241.
- GAWRILENKO, A. 1924 Die anomale Entwicklung des Zentralnervensystems bei einem Hühnerembryo. Anat. Anz., Bd. 59, S. 12-31.
- GRÄPER, L. 1926 Die frühe Entwicklung des Hühnchens nach Kinaufnahmen des lebenden Embryo. Anat. Anz., Bd. 61 (Erganz.), S. 54-57.
- 1927 Zur Schwanzknospen und Primitiventwicklung des Hühnchens. Anat. Anz., Bd. 62, S. 398-399.

- HOADLEY, L. 1926 Developmental potencies of parts of the early blastoderm of the chick. III. The nephros, with special reference to the pro- and mesonephric portions. *J. Exp. Zool.*, vol. 43, pp. 197-223.
- HOLMDAHL, D. E. 1925 Experimentelle Untersuchungen über die Lage der Grenze zwischen primärer und sekundärer Körperentwicklung beim Huhn. *Anat. Anz.*, Bd. 59, S. 393-396.
- 1925 Die erste Entwicklung des Körpers bei den Vögeln und Säugetieren, inkl. dem Menschen, besonders mit Rücksicht auf die Bildung des Rückenmarks, des Zöloms und der entodermalen Kloake nebst einem Exkurs über die Entstehung der Spina bifida in der Lumbosakralregion. I. Gegenbaur's *Morph. Jahrbuch*, Bd. 54, S. 333-384. II-V. *Ibid.*, Bd. 55, S. 112-208.
- 1926 Über die Entstehung der kaudalen Körperpartie. *Anat. Anz.*, Bd. 62, S. 180-189.
- HUNT, T. E. 1929 a Hensen's node as an organizer in the formation of the chick embryo. *Anat. Rec.*, vol. 42, p. 22.
- 1929 b An experimental study of Hensen's node in the chick embryo. *Proc. Soc. Exp. Biol. and Med.*, vol. 27, pp. 84-86.
- 1931 An experimental study of the independent differentiation of the isolated Hensen's node and its relation to the formation of axial and non-axial parts in the chick embryo. *J. Exp. Zool.*, vol. 59, pp. 395-428.
- HYMAN, L. H. 1927 The metabolic gradients of vertebrate embryos. III. The chick. *Biol. Bull.*, vol. 52, pp. 1-39.
- KEIBEL, F., AND C. ELZE 1908 Normentafel zur Entwicklungsgeschichte des Menschen, S. 85. Jena.
- LILLIE, F. R. 1904 Experimental studies on the development of organs in the embryo of the fowl. *Biol. Bull.*, vol. 7, pp. 33-54.
- 1908 The development of the chick. Henry Holt & Co., New York.
- SCHREINER, K. E. 1902 Über die Entwicklung der Amniotenniere. *Zeitschr. f. wiss. Zool.*, Bd. 71, S. 1-188.
- SCHUMACHER, S. 1927 Über die sogenannte Viervielfachung des Medullarrohres (bzw. des Canalis centralis) bei Embryonen. *Zeitschr. f. mikr.-anat. Forsch.*, Bd. 10, S. 75-109.
- WAELSCH, L. 1914 Über experimentelle Erzeugung von Epithelwucherungen und Vervielfachungen des Medullarrohres bei Hühnerembryonem. *Arch. f. Entw.*, Bd. 38, S. 509-539.
- WETZEL, R. 1929 Untersuchungen am Hühnchen. Die Entwicklung des Keims während der ersten beiden Bruttage. *Arch. f. Entw.*, Bd. 119, S. 188-321.
- WILLIER, B. H. 1924 The endocrine glands and the development of the chick. I. The effects of thyroid grafts. *Am. J. Anat.*, vol. 33, pp. 67-103.
- 1926 The development of implanted chick embryos following the removal of the 'primordial germ cells.' *Anat. Rec.*, vol. 34, p. 158.
- 1930 A study of the origin and differentiation of the suprarenal gland in the chick embryo by chorio-allantoic grafting. *Phys. Zool.*, vol. 3, pp. 201-225.
- WILLIER, B. H., AND MARY E. RAWLES 1931 The relation of Hensen's node to the differentiating capacity of whole chick blastoderms as studied in chorio-allantoic grafts. *J. Exp. Zool.*, vol. 59, pp. 429-465.

